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Selective Baeyer–Villiger oxidation of racemic ketones in aqueous–organic media catalyzed by phenylacetone monooxygenase

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Abstract—The enantioselective kinetic resolution of a set of racemic substituted 3-phenylbutan-2-ones employing phenylacetone monooxygenase (PAMO) in non-conventional media was performed. The studies have revealed the effects of a range of solvents on the biocatalytic properties of the biocatalyst. Also, the enzymatic oxidation of α-acetylphenylacetonitrile was performed using organic cosolvents. This has resulted in a dynamic kinetic resolution of this cyanoketone yielding enantiopure (*R*)-2-acetoxyphenylacetonitrile with moderate yields depending on the reaction conditions employed.

1. Introduction

Chiral ketones as well as optically active esters and lactones are organic molecules that are of interest for use in chemical synthesis. Since its discovery more than 100 years ago,[1] the Baeyer–Villiger oxidation has represented a very useful tool for the preparation of these high-added value compounds.[2] This reaction involves the oxidative cleavage of a carbon–carbon bond adjacent to a carbonyl function and is carried out chemically with peracids or with peroxides and a Lewis acid. Unfortunately, the use of such strong oxidants presents several drawbacks. To overcome these limitations, some 'greener' procedures have been developed in recent years, for example, the use of organocatalytic compounds with mild oxidants[3] or the employment of enzymes as chiral catalysts.[4]

Baeyer–Villiger monooxygenases are oxidoreductases that catalyze a set of oxidative reactions, including the oxidation of aliphatic and cyclic ketones into esters and lactones, respectively, using molecular oxygen.[5] Due to the mild reaction conditions employed and the high regio- and/or enantioselectivities obtained, BVMOs represent a valuable alternative for the chemical Baeyer–Villiger oxidation to synthesize chiral esters and lactones.

Some years ago, phenylacetone monooxygenase (PAMO) from *Thermobifida fusca* was first reported as a Baeyer–Villiger monooxygenase.[6] This biocatalyst is NADPH dependent, monomeric, thermostable and has been successfully employed with high selectivity in the preparation of chiral esters and sulfoxides.[7] It has also been shown that it can oxidize amines and boron-containing compounds. PAMO exhibits a narrow substrate specify but some mutants have been prepared to overcome this drawback.[8]

Enzymes perform their function in aqueous buffer, but it has been shown that some of them can act as catalysts in organic solvents with low water contents.[9] Recently, it has been discovered that certain isolated BVMOs can perform oxidation when working in non-conventional media (mixtures of aqueous buffer with miscible or immiscible organic solvent).[10] For all cosolvents tested, a decrease in the enzymatic activity and stability was observed. The best

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results were obtained when working in mixtures of buffer and 10% methanol. It was also discovered that the addition of certain water-miscible cosolvents such methanol or ethanol when employing PAMO or ethionamide monooxygenase (EtaA)[11] led to a significant increase and in some cases to a reversal in the enzyme enantioselectivity for the enzymatic oxidation of phenyl sulfides to the corresponding chiral sulfoxides. Wild type PAMO and some derived mutant enzymes have been employed in the enantioselective Baeyer–Villiger oxidation of several ketones on a preparative scale when working in biphasic (aqueous buffer–organic cosolvent) systems.[12]

In the present study, we report on the enantioselective Baeyer–Villiger oxidation of racemic benzylketones catalyzed by PAMO when employing non-conventional media.

2. Results and discussion

2.1. PAMO-catalyzed oxidation of 3-phenylbutan-2-ones in aqueous–organic media

Our initial experiments were dedicated to investigate the effect of a broad range of organic solvents, presenting different physico-chemical properties, on the activity and selectivity of PAMO when this enzyme catalyzed the Baeyer–Villiger oxidation of a racemic substituted 3-phenylbutan-2-one, as shown in Scheme 1. Purified PAMO is effective in the oxidation of molecules with this structure, as described in previous reports.[7c] Two different situations were observed when the enzymatic reactions were carried out in the solvents tested: the use of water-miscible organic solvents resulted in a one-phase system, while the water-immiscible solvents led to a biphasic system. In the latter case, the enzyme and the hydrophilic components of the mixture will be mainly found in the aqueous layer whereas the substrate will be present mostly in the organic phase. All the reactions were carried out using a 50 mM Tris/HCl buffer at pH 8.0 as the aqueous phase and were conducted using the isolated enzyme. As a consequence, a second ancillary enzymatic system (glucose-6-phosphate with glucose-6-phosphate dehydrogenase) was included in the reaction mixture to regenerate the NADPH coenzyme.[13]

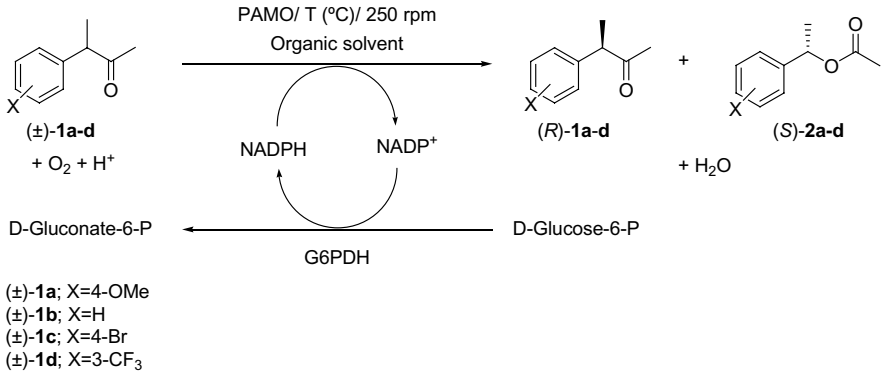
The enzymatic oxidation of (±)-3-(4-methoxyphenyl)butan-2-one **1a** in buffer alone led to (*S*)-**2a** ($c = 31\%$ after 1 h) with a moderate enantioselectivity ($E = 32$, Table 1, entry 1).[14] When the Baeyer–Villiger oxidation of (±)-**1a** was conducted in buffer with 30% of cosolvents displaying a high hydrophilic character, for example, DMSO and DMF, a loss in enzymatic activity was observed, as shown in entries 2 and 3. Nevertheless, the cosolvents only marginally affected the enantioselectivity. As previously found in the enzymatic oxidation of organic sulfides,[10] the presence of 30% methanol in the reaction medium had an important effect on the enzymatic selectivity. Again, a significant increase in the PAMO enantioselectivity was achieved: (±)-**1a** was oxidized to (*S*)-**2a**, thereby yielding the (*R*)-ketone with $E = 49$, in a process slower than in buffer only ($c = 13\%$ after 3 h).

For the rest of the solvents tested in a 30% concentration (entries 4–12), low PAMO activities were achieved. When

Table 1. Effect of organic cosolvents and methanol concentration on PAMO-catalyzed oxidation of (±)-3-(4-methoxyphenyl)butan-2-one, **1a**[a]

Entry	Cosolvent	*t* (h)	ee[b] (%) (*R*)-**1a**	ee[b] (%) (*S*)-**2a**	*c*[c] (%)	*E*[d]
1	None	1	44	91	31	32
2	30% DMSO	3	23	90	20	24
3	30% DMF	3	7	93	7	30
4	30% MeOH	3	14	95	13	49
5	30% AcCN	6	n.d.	n.d.	⩽1.0	n.d.
6	30% Dioxane	3	60	50	54	5.5
7	30% EtOH	3	6	13	31	1.5
8	30% *i*-PrOH	3	39	54	42	5.0
9	30% AcOEt	4	20	86	19	16
10	30% *t*-BuOMe	4	31	53	39	4.0
11	30% *i*-Pr_2O	4	17	61	22	5.0
12	30% Toluene	10	n.d.	n.d.	⩽1.0	n.d.
13	30% Hexane	4	54	79	40	14
14	10% MeOH	1	21	94	18	43
15	50% MeOH	8	15	95	13	46
16	70% MeOH	8	n.d.	n.d.	⩽1.0	n.d.
17	90% MeOH	8	n.d.	n.d.	⩽1.0	n.d.

n.d. not determined.
[a] For reaction conditions see Section 4.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Enantiomeric ratio, $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.



Scheme 1. PAMO-catalyzed oxidation of substituted 3-phenylbutan-2-ones in aqueous–organic media.

employing 30% acetonitrile and toluene, no oxidation was observed. Concerning the selectivity of the reaction, a considerable loss in the E values was also observed. The highest enantioselectivities when using water-immiscible cosolvents were measured in 30% ethyl acetate and hexane (entries 9 and 13, $E = 16$–14, respectively). PAMO almost completely lost its selectivity when carrying out the oxidations in any other solvent, both working in a monophasic or a biphasic system.

To study the effect of cosolvent concentration, the effect of different amounts of methanol was analyzed. As shown in Table 1 (entries 14–17), the addition of higher contents of methanol produced a gradual inactivation of the enzyme. No reaction was observed when working at methanol concentrations of 70% and 90%, but it is interesting to notice that PAMO can still work at 50% MeOH contents, yielding a conversion of 13% after 8 h. For all the MeOH concentrations in which the Baeyer–Villiger oxidation of (±)-**1a** was observed, better E values were achieved than in buffer alone, demonstrating that even at moderate concentrations, methanol is able to improve the selectivity of PAMO.

Once it was established that methanol was the best cosolvent for the enzymatic oxidation of (±)-**1a** in terms of selectivity, the study was extended to other 3-phenylbutan-2-ones, as shown in Table 2. As previously reported, the enzymatic oxidation of racemic 3-phenyl-2-butanone **1b** catalyzed by PAMO was a process with a high enantioselectivity ($E = 188$) leading to (*R*)-**1b** and (*S*)-**2b** with a conversion of 27% after 1 h (entry 1). The addition of 10% or 30% methanol to the reaction medium did not alter the selectivity of the resolution, yielding excellent E values with a slight decrease in the enzymatic activity.

Table 2. Effect of methanol (10% and 30% v v^{-1}) on PAMO-catalyzed oxidation of substituted 3-phenylbutan-2-ones[a]

Entry	Ketone	Cosolvent	ee[b] (%) (*R*)-ket	ee[b] (%) (*S*)-est	c[c] (%)	E
1	(±)-**1b**	None	36	98	27	188
2	(±)-**1b**	10% MeOH	20	99	17	174
3	(±)-**1b**	30% MeOH	24	99	20	179
4	(±)-**1c**	None	14	44	24	3.0
5	(±)-**1c**	10% MeOH	22	73	23	7.9
6	(±)-**1c**	30% MeOH	24	83	22	14
7	(±)-**1d**	None	47	43	52	4.5
8	(±)-**1d**	10% MeOH	42	52	45	5.0
9	(±)-**1d**	30% MeOH	60	69	46	10

[a] For reaction conditions see Section 4. Reactions were stopped after 1 h.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.

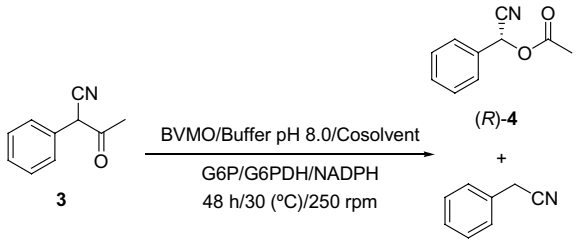
When PAMO was employed as biocatalyst in the enzymatic oxidation of a 3-phenylbutanone presenting an electron-withdrawing group in the *para*-position of the aromatic ring as (±)-**1c**, compounds (*S*)-**2c** and (*R*)-**1c** were obtained in a resolution with a low E value ($E = 3.0$, Table 2, entry 4). Increasing amounts of methanol did not alter the PAMO activity, but had a profound effect on the enantioselectivity of the oxidation: it was possible to obtain an E value of 7.9 when working at 10% methanol and $E = 14$ at 30% MeOH, the latter being almost five times more enantioselective than in the absence of the organic solvent.

The same effect was observed in the PAMO-catalyzed Baeyer–Villiger oxidation of a *meta*-substituted benzylketone such (±)-3-(3-trifluoromethylphenyl)butan-2-one (**1d**). When the reaction was performed at 30 °C in Tris/HCl buffer pH 8.0, (*S*)-**2d** was obtained with a 52% conversion in a process of low enantioselectivity (entry 7). Addition of 10% or 30% methanol led to a slight loss in the PAMO activity (entries 8 and 9) and a significant increase in the E value ($E = 10$ at 30% MeOH), confirming the positive effect of methanol on the enantioselectivity of this enzyme.

2.2. Enzymatic oxidation of α-acetylphenylacetonitrile catalyzed by PAMO

To probe the biocatalytic potential of PAMO for dynamic kinetic resolutions, the conversion of α-acetylphenylacetonitrile **3** was studied. For this oxidation reaction, it is important to note that cyanoketone **3** presents a hydrogen atom with a very acidic character. As a result, this compound readily racemizes. For this reason, chiral **4** could be obtained in high yields and high enantiomeric purity in a dynamic kinetic resolution process. When the enzymatic Baeyer–Villiger reaction of α-acetylphenylacetonitrile was performed in 50 mM Tris/HCl at different pHs and temperatures, no oxidation product **4** was observed even after long reaction times. Only the side byproduct phenylacetonitrile **5** was observed, as shown in Table 3. This compound was also found in the blank reactions performed in the absence of biocatalyst and corresponds to the hydrolysis product of α-acetylphenylacetonitrile in basic medium. The formation of **5** was faster at higher pHs and tempera-

Table 3. Effect of different cosolvents on PAMO-catalyzed oxidation of α-acetylphenylacetonitrile **3**[a]



Entry	Cosolvent	**3**[b] (%)	(*R*)-**4**[b] (%)	**5**[b] (%)	ee[b] (%) (*R*)-**4**
1	10% DMSO	60	7.0	33	⩾98
2	10% MeOH	90	7.0	3.0	96
3	10% Dioxane	80	6.0	15	97
4	10% AcOEt	70	22	8.0	⩾98
5	10% i-Pr_2O	76	13	11	⩾98
6	10% t-BuOMe	79	8.0	13	⩾98
7	10% CH_2Cl_2	95	2.0	3.0	⩾98
8	10% Toluene	92	2.0	6.0	⩾98
9	10% Hexane	91	5.0	4.0	⩾98

[a] [**3**] = 2.0 mg mL^{-1}. Reactions were carried out employing 0.5 units of PAMO.
[b] Determined by GC.

tures. To confirm this, when **3** was dissolved in a 0.5 M sodium hydroxide solution at room temperature, **5** was the only product observed after a few minutes.

In view of these results, the oxidation was performed in a one-phase system of aqueous buffer and methanol. Several experiments were carried out at different concentrations, temperatures and pHs. Under all the conditions, it was possible to obtain some enantiopure oxidation product (*R*)-**4**, but with low conversions after 2 days reaction time. Also, the presence of methanol led to the formation of the side product **5**. Entry 2 of Table 3 shows the best oxidation achieved with this cosolvent (10% methanol, pH 8.0 and 30 °C led to 7.0% of (*R*)-**4** after 48 h).

We decided to study the effect of other organic cosolvents with different properties in the PAMO-catalyzed oxidation of **3** (Table 3). For all the reaction media tested, some phenylacetonitrile **5** was formed, even when performing the reactions without biocatalyst. As shown in Table 3, oxidations were carried out in buffer pH 8.0 containing 10% organic solvent and stopped after 48 h. Compound (*R*)-**4** was obtained with high enantiomeric excess (ee $\geqslant$ 96%) in all conditions. The presence of 10% cosolvent led, in most cases to low yields of enantiopure **4** (lower than 10%), the formation of higher quantities of byproduct **5** (see entries 1 and 3, employing DMSO or 1,4-dioxane) and the recovery of high amounts of the starting ketone. Only when working with two water immiscible solvents, that is, *i*-Pr_2O or AcOEt, was the formation of 2-acetoxyphenylacetonitrile was higher than 10% after 48 h, especially in the case of AcOEt (22%, entry 4).

As *i*-Pr_2O and AcOEt were the best cosolvents for the Baeyer–Villiger oxidation, certain reaction parameters were modified to study their effect on PAMO properties. In these reactions, the amount of biocatalyst was increased to obtain higher conversions (Table 4). As shown in entry 1, a 10% *i*-Pr_2O at pH 8 and 30 °C allowed us to obtain enantiopure (*R*)-**4** in about 30% after 48 h. When increasing the cosolvent amount to 20%, the oxidation became much slower (only 6.0% of enzymatic product formed). When the Baeyer–Villiger oxidation was performed at pH 9.0, **4** was obtained with a slight decrease in enzyme selectivity (ee = 93%) and good yield, but the side product was formed to a much higher extent than at lower pH (entry 3). Ethyl acetate was a better cosolvent for this process. When using 1.0 unit of PAMO at pH 8 and 30 °C, it was possible to obtain 48% of enantiopure (*R*)-2-acetoxyphenylacetonitrile after 48 h and 58% after 72 h. These data confirm that the reaction involves a dynamic kinetic resolution. Longer reactions times did not increase the amount of oxidation product to a great extent.

The effects of temperature and pH of this enzymatic process were also studied (Table 4). Performing the reaction at 20 °C led to **4** in a high yield, and with only a minor quantity of **5**. On the contrary, oxidation of **3** at 40 °C formed phenylacetonitrile in 62%, with only 4% of (*R*)-**4**. Carrying out the conversion at a higher pH resulted in a decrease in PAMO activity and selectivity (entry 9), while at pH 7.5 almost all the starting material was recovered after 48 h (entry 8). In addition, the ethyl acetate concentration was also analyzed as a key parameter in the enzymatic oxidation of **3**. When working in 5% AcOEt, 25% of **4** was formed with ee = 94% and also phenylacetonitrile was synthesized in the same amount. The highest PAMO activity in the formation of enantiopure **4** was observed at 10% AcOEt. An increase in the cosolvent concentration led to a progressive decrease in the enzymatic activity (entries 11–13) and in the formation of **5**. When performing the oxidation in 50% AcOEt, no conversion was observed. Concerning the biocatalyst selectivity, enantiopure (*R*)-2-acetoxyphenylacetonitrile was synthesized until 40% cosolvent concentration. As we were working in a biphasic buffer–ethyl acetate system, 2.0 equiv of a phase transfer reagent as Bu_4NHSO_4 was added in the enzymatic oxidation performed at pH 8.0 and 30 °C. The presence of this compound led to a total loss in the enzymatic activity, as shown in entry 14.

The influence of **3** concentration on the activity and selectivity of PAMO was studied when conducting the oxidations in buffer with 10% AcOEt (Fig. 1). After 42 h, at all

Table 4. Enzymatic oxidation of α-acetylphenylacetonitrile **3** employing *i*-Pr_2O and AcOEt as cosolvents[a]

Entry	Cosolvent	pH	*T* (°C)	*t* (h)	**3**[b] (%)	(*R*)-**4**[b] (%)	**5**[b] (%)	ee (*R*)-**4**[b] (%)
1	10% *i*-Pr_2O	8.0	30	48	66	30	4.0	$\geqslant$98
2	20% *i*-Pr_2O	8.0	30	48	87	6.0	7.0	$\geqslant$98
3	10% *i*-Pr_2O	9.0	30	48	63	26	11	93
4	10% AcOEt	8.0	30	48	40	48	12	$\geqslant$98
5	10% AcOEt	8.0	30	72	28	58	14	$\geqslant$98
6	10% AcOEt	8.0	20	48	41	56	3	$\geqslant$98
7	10% AcOEt	8.0	40	48	33	5.0	62	$\geqslant$98
8	10% AcOEt	7.5	30	48	93	3.0	4.0	$\geqslant$98
9	10% AcOEt	9.0	30	48	59	17	24	95
10	5% AcOEt	8.0	30	48	52	25	23	94
11	20% AcOEt	8.0	30	48	62	35	3.0	$\geqslant$98
12	30% AcOEt	8.0	30	48	83	15	2.0	$\geqslant$98
13	40% AcOEt	8.0	30	48	83	15	2.0	95
14[c]	10% AcOEt	8.0	30	48	89	$\leqslant$1.0	10	—

[a] [**3**] = 2.0 mg mL^{-1}. For reaction conditions see Section 4. 1 unit of PAMO was employed.
[b] Determined by GC.
[c] Reaction performed with 2 equiv of Bu_4NHSO_4 as phase transfer reagent.

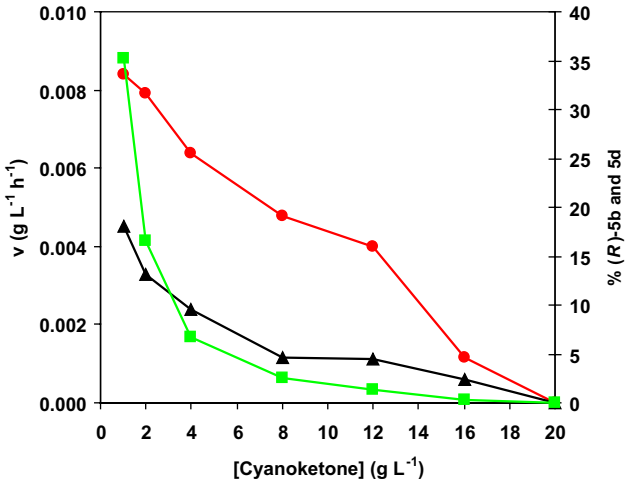


Figure 1. Effect of α-acetylphenylacetonitrile concentration on the formation of (*R*)-**4** (green line), **5** (black line) and on the reaction rate (red line) in PAMO-catalyzed oxidation of **3** in 50 mM Tris/HCl pH 8.0, 10% AcOEt at 30 °C.

the concentrations tested (1–20 g L^{-1}), enantiopure (*R*)-2-acetoxyphenylacetonitrile was obtained. This shows that substrate concentration does not affect the enzyme selectivity.

As expected, when the concentrations were higher, enantiopure (*R*)-**4** was obtained in lower quantities and also the formation of the side product **5** was slower at high concentrations. It is interesting to notice that at substrate concentration of 1 g L^{-1} **4** was formed in larger quantities than **5** (ratio 2:1), while by increasing the substrate concentration the side product was synthesized to a higher extent than the oxidation one (at 16 g L^{-1} the ratio of **4**:**5** was 1:8), indicating a decrease in the PAMO activity. The reaction rates (expressed as grams of **3** consumed per liter and per hour) were also studied, yielding a maximum value at low concentrations (1–2 g L^{-1}). Higher ketone amounts in the medium decreased the reaction rate. When working at 20 g L^{-1} no oxidation was observed.

3. Conclusion

The addition of different amounts of organic cosolvents to the reaction medium in enzymatic Baeyer–Villiger oxidation of racemic ketones catalyzed by PAMO can improve the biocatalytic properties of this biocatalyst. Herein, two different effects can be discriminated: (1) an increase of the enantioselectivity in the resolution of different substituted 3-phenylbutan-2-ones by different concentrations of methanol, up to a 50% v v^{-1} and, (2) higher conversions in the PAMO-catalyzed oxidation of α-acetylphenylacetonitrile due to an increase of the substrate solubility in the reaction medium when performing the reactions. For the latter substrate, it was possible to perform a dynamic kinetic resolution, achieving enantiopure (*R*)-2-acetoxyphenylacetonitrile with good conversions in 50 mM Tris/HCl pH 8.0 at 20 °C containing 10% ethyl acetate. So, by choosing the reaction parameters properly, such as temperature, pH, the type and concentration of organic cosolvent when performing oxidations catalyzed by PAMO, it is possible to obtain better results in terms of selectivity and conversion.

4. Experimental

4.1. General

Recombinant histidine-tagged phenylacetone monooxygenase (PAMO) was obtained as previously described.[6] One unit of BVMO will oxidize 1.0 μmol of phenylacetone to benzyl acetate per minute at pH 8 and 30 °C in the presence of NADPH. Glucose-6-phosphate dehydrogenase (170 U/mg) from *Leuconostoc mesenteroides* was obtained from Fluka-BioChemika. All reagents and solvents employed were of the highest quality grade available and were from Sigma–Aldrich–Fluka and Acros Organics.

The racemic ketones (±)-**1a**–**d** were prepared according to the literature, using methyl iodide and NaOH in a biphasic medium water/dichloromethane (yields from 20% to 80%, depending on the substrate).[15] Acetate (±)-**2a** was prepared by direct oxidation with *m*-CPBA/dichloromethane with 75% yield, while racemic esters (±)-**2b**–**d** and (±)-**4** were synthesized by chemical acetylation of the corresponding alcohols (yields from 40% to 90%).

Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F_{254} plates and visualized by UV irradiation. Flash chromatography was carried out with silica gel 60 (230–240 mesh, Merck). IR spectra were recorded on a Perkin–Elmer 1720-X infrared Fourier transform spectrophotometer using KBr pellets. Optical rotations were measured using a Perkin–Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR, ^{13}C NMR, and DEPT spectra were with TMS (tetramethylsilane) as the internal standard with Bruker AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.4 MHz) and Bruker AC-300-DPX (^{1}H, 300.13 MHz and ^{13}C, 75.4 MHz) spectrometers. The chemical shift values (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). $APCI^+$ using a Hewlett Packard 1100 chromatograph mass detector or EI^+ with a Hewlett Packard 5973 mass spectrometer was used to record mass spectra (MS).

GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with the following columns: Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1.0 bar N_2) and Krompex Cydex B (25 m × 0.25 mm × 0.25 μm, 1.0 bar N_2). For all the analyses, the injector temperature is 225 °C and the FID temperature is 250 °C.

The absolute configuration of ester **2a** was determined by comparison of the GC chromatograms with the patterns described in previous experiments for the known configuration.[16] For esters **2b**–**d**, the absolute configuration was established by comparison with an authentic sample prepared from chemical acylation of the corresponding commercial chiral (*S*)-alcohols. Absolute configuration of **4** was determined in the same way, starting in this case from the commercial alcohol of (*R*)-configuration.

4.2. General procedure for the PAMO-catalyzed oxidation of racemic ketones (±)-1a–d and 3

Unless otherwise stated, the corresponding racemic ketones (±)-**1a**–**d** and **3** (0.02 mmol, 1.0 equiv) were dissolved in 50 mM Tris/HCl at different pHs/organic cosolvent system (1.0 mL), containing glucose-6-phosphate (0.04 mmol, 2.0 equiv), glucose-6-phosphate dehydrogenase (10.0 units), NADPH (0.2 mM) and the Baeyer–Villiger monooxygenase (1.0 unit). The mixture was shaken at 250 rpm and the selected temperature in a rotatory shaker for the times indicated. The reaction was then stopped, worked up by extraction with ethyl acetate (3 × 0.5 mL), dried over Na_2SO_4 and analyzed directly by chiral GC to determine the conversion and the enantiomeric excesses of the remaining ketones (*R*)-**1a**–**d**, **3** and the esters **2a**–**d** of (*S*)-configuration and (*R*)-**4**. In the case of ketones (±)-**1a**–**d**, for all the solvents tested, control experiments in the absence of enzyme resulted in no conversion. For **3**, the reactions performed without PAMO showed **5** as undesired byproduct.

4.3. Enzymatic oxidation catalyzed by PAMO of racemic ketones (±)-1a, (±)-1c and (±)-1d at multimilligram scale in buffer containing 30% MeOH

Racemic ketones (±)-**1a**, (±)-**1c** and (±)-**1d** (0.5 mmol) were dissolved in 20 mL of Tris/HCl buffer (50 mM, pH 8.0), containing a 30% MeOH for substrates **1c** and **1d**, glucose-6-phosphate (1.0 mmol), glucose-6-phosphate dehydrogenase (40 units), NADPH (0.8 mM) and PAMO (4 units). The mixtures were shaken at 20 °C and 250 rpm for 72 h and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude residues were purified by flash chromatography on silica gel employing as eluent hexane/diethyl ether 8:2, to afford (*R*)-**1a** (46.7 mg, 52% yield); (*S*)-**2a** (23.0 mg, 24% yield); (*R*)-**1c** (75.4 mg, 67% yield); (*S*)-**2c** (17.1 mg, 14% yield); (*R*)-**1d** (46.4 mg, 43% yield) and (*S*)-**2d** (42.8 mg, 37% yield).

4.3.1. (*R*)-3-(4-Methoxyphenyl)butan-2-one, (*R*)-1a. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.36 (d, 3H, $^3J_{HH}$ 7.0 Hz), 2.03 (s, 3H), 3.68 (q, 1H, $^3J_{HH}$ 6.9 Hz), 3.79 (s, 3H), 6.87 (d, 2H$_{ar}$, $^3J_{HH}$ 6.7 Hz), 7.13 (d, 2H$_{ar}$, $^3J_{HH}$ 6.8 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.1 (CH_3), 28.1 (CH_3), 52.8 (CH), 55.2 (CH_3), 114.3 (2CH$_{ar}$), 128.7 (2CH$_{ar}$), 132.6 (C$_{ar}$), 158.7 (C$_{ar}$), 209.1 (C=O). MS (EI$^+$, *m*/*z*): 178 (M$^+$, 35%), 135 (100%). Determination of the ee by GC analysis: Cydex B, 90 °C (30 min), 5 °C/min, 120 °C (30 min), 10 °C/min, 200 °C; t_R (*R*) 68.0 min; t_R (*S*) 68.5 min. $[\alpha]_D^{25} = -22.7$ (*c* 1.12, $CHCl_3$), ee 44%.

4.3.2. (*R*)-1-(4-Methoxyphenyl)ethyl acetate, (*S*)-2a. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.54 (d, 3H, $^3J_{HH}$ 6.6 Hz), 2.07 (s, 3H), 3.82 (s, 3H), 5.87 (q, 1H, $^3J_{HH}$ 6.6 Hz), 6.90 (d, 2H$_{ar}$, $^3J_{HH}$ 8.5 Hz), 7.32 (d, 2H$_{ar}$, $^3J_{HH}$ 8.8 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.9 (CH_3), 21.4 (CH_3), 54.7 (CH_3), 71.5 (CH), 113.3 (2CH$_{ar}$), 127.1 (2CH$_{ar}$), 133.2 (C$_{ar}$), 158.8 (C$_{ar}$), 169.9 (C=O). MS (EI$^+$, *m*/*z*): 194 (M$^+$, 48%), 134 (100%). Determination of the ee by GC analysis: Cydex B, 90 °C (30 min), 5 °C/min, 120 °C (30 min), 10 °C/min, 200 °C; t_R (*R*) 69.0 min; t_R (*S*) 69.5 min.

4.3.3. (*R*)-3-Phenylbutan-2-one, (*R*)-1b. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.39 (d, 3H, $^3J_{HH}$ 7.0 Hz), 2.12 (s, 3H), 3.75 (q, 1H, $^3J_{HH}$ 7.0 Hz), 7.19–7.39 (m, 5H$_{ar}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.1 (CH_3), 28.3 (CH_3), 53.6 (CH), 127.1 (CH$_{ar}$), 127.7 (2CH$_{ar}$), 128.9 (2CH$_{ar}$), 140.5 (C$_{ar}$), 208.8 (C=O). MS (APCI$^+$, *m*/*z*): 149 [(M+H)$^+$, 100%]. Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C (5 min); t_R (*R*) 44.3 min; t_R (*S*) 46.1 min.

4.3.4. (*S*)-1-Phenyethyl acetate, (*S*)-2b. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.56 (d, 3H, $^3J_{HH}$ 6.6 Hz), 2.10 (s, 3H), 5.91 (q, 1H, $^3J_{HH}$ 6.6 Hz), 7.28–7.39 (m, 5H$_{ar}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.9 (CH_3), 21.7 (CH_3), 71.8 (CH), 125.6 (2CH$_{ar}$), 127.4 (CH$_{ar}$), 128.0 (2CH$_{ar}$), 141.2 (C$_{ar}$), 169.8 (C=O). MS (APCI$^+$, *m*/*z*): 165 [(M+H)$^+$, 100%]. Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C (5 min). t_R (*S*) 42.7 min; t_R (*R*) 50.1 min.

4.3.5. (*R*)-3-(4-Bromophenyl)butan-2-one, (*R*)-1c. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.37 (d, 3H, $^3J_{HH}$ 6.9 Hz), 2.05 (s, 3H), 3.71 (q, 1H, $^3J_{HH}$ 6.9 Hz), 7.08 (d, 2H, $^3J_{HH}$ 8.6 Hz), 7.45 (d, 2H, $^3J_{HH}$ 8.6 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.2 (CH_3), 28.4 (CH_3), 53.1 (CH), 121.2 (C$_{ar}$), 129.5 (2CH$_{ar}$), 132.1 (2CH$_{ar}$), 139.5 (C$_{ar}$), 208.2 (C=O). MS (EI$^+$, *m*/*z*): 226 (M$^+$, 21%), 183 (92%), 104 (100%). Determination of the ee by GC analysis: RtβDEXse, 100 °C (5 min), 3 °C/min, 150 °C (5 min), 10 °C/min, 200 °C; t_R (*R*) 56.6 min; t_R (*S*) 58.2 min. $[\alpha]_D^{25} = -17.1$ (*c* 1.50, $CHCl_3$), ee 24%.

4.3.6. (*S*)-1-(4-Bromophenyl)ethyl acetate, (*S*)-2c. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.51 (d, 3H, $^3J_{HH}$ 6.6 Hz), 2.06 (s, 3H), 5.82 (q, 1H, $^3J_{HH}$ 6.6 Hz), 7.23 (2H$_{ar}$, $^3J_{HH}$ 8.4 Hz), 7.47 (2H$_{ar}$, $^3J_{HH}$ 8.5 Hz). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (CH_3), 22.0 (CH_3), 71.5 (CH), 121.6 (C$_{ar}$), 127.7 (2CH$_{ar}$), 131.5 (2CH$_{ar}$), 140.6 (C$_{ar}$), 170.1 (C=O). MS (EI$^+$, *m*/*z*): 242 (M$^+$, 32%), 184 (79%), 104 (73%). Determination of the ee by GC analysis: RtβDEXse, 100 °C (5 min), 3 °C/min, 150 °C (5 min), 10 °C/min, 200 °C; t_R (*S*) 54.1 min; t_R (*R*) 57.6 min.

4.3.7. (*R*)-3-(3-Trifluoromethylphenyl)butan-2-one, (*R*)-1d. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 1.43 (d, 3H, $^3J_{HH}$ 7.1 Hz), 2.08 (s, 3H), 3.83 (q, 1H, $^3J_{HH}$ 7.0 Hz), 7.39–7.55 (m, 4H$_{ar}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.3 (CH_3), 28.5 (CH_3), 53.3 (CH), 123.9 (CF_3, $^1J_{CF}$ 270.6 Hz), 124.1 (CH$_{ar}$, $^3J_{CF}$ 3.7 Hz), 124.6 (CH$_{ar}$, $^3J_{CF}$ 3.7 Hz), 129.4 (CH$_{ar}$), 131.1 (CH$_{ar}$), 131.2 (C$_{ar}$, $^2J_{CF}$ 32.1 Hz), 141.4 (C$_{ar}$), 207.8 (C=O). MS (EI$^+$, *m*/*z*): 216 (M$^+$, 16%), 173 (40%). Determination of the ee by GC analysis: RtβDEXse, 100 °C (20 min), 2 °C/min, 150 °C (5 min), 10 °C/min, 200 °C; t_R (*R*) 21.7 min; t_R (*S*) 23.7 min. $[\alpha]_D^{25} = -38.7$ (*c* 1.15, $CHCl_3$), ee 60%.

4.3.8. (*S*)-1-(3-Trifluoromethylphenyl)ethyl acetate, (*S*)-2d. Colorless oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ

1.55 (d, 3H, $^3J_{HH}$ 6.8 Hz), 2.10 (s, 3H), 5.91 (q, 1H, $^3J_{HH}$ 6.6 Hz), 7.47–7.61 (m, 4H$_{ar}$). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (CH_3), 22.2 (CH_3), 71.5 (CH), 122.7 (CH_{ar}, $^3J_{CF}$ 3.7 Hz), 124.0 (CF_3, $^1J_{CF}$ 270.6 Hz), 124.6 (CH_{ar}, $^3J_{CF}$ 3.7 Hz), 128.9 (CH_{ar}), 129.5 (CH_{ar}), 130.9 (C_{ar}, $^2J_{CF} = 32.1$ Hz), 142.7 (C_{ar}), 170.1 (C=O). MS (EI^+, m/z): 232 (M^+, 11%), 190 (90%). Determination of the ee by GC analysis: RtβDEXse, 100 °C (20 min), 2 °C/min, 150 °C (5 min), 10 °C/min, 200 °C; t_R (*S*) 18.3 min; t_R (*R*) 22.8 min. $[\alpha]_D^{25} = +78.7$ (*c* 0.95, $CHCl_3$), ee 69%.

4.3.9. (*R*)-2-Acetoxyphenylacetonitrile, (*R*)-4. Pale yellow oil. ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.22 (s, 3H), 6.46 (s, 1H), 7.49–7.58 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 20.4 (CH_3), 68.8 (CH), 116.0 (CN), 127.8 (2CH_{ar}), 129.2 (2CH_{ar}), 130.3 (CH_{ar}), 131.6 (C_{ar}), 168.9 (C). MS (EI^+, m/z): 175 (M^+, 35%), 133 (98%), 115 (97%). Determination of the ee by GC analysis: RtβDEXse, 130 °C (1 min), 2 °C/min, 200 °C (5 min). t_R (*S*) 15.2 min; t_R (*R*) 17.0 min.

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